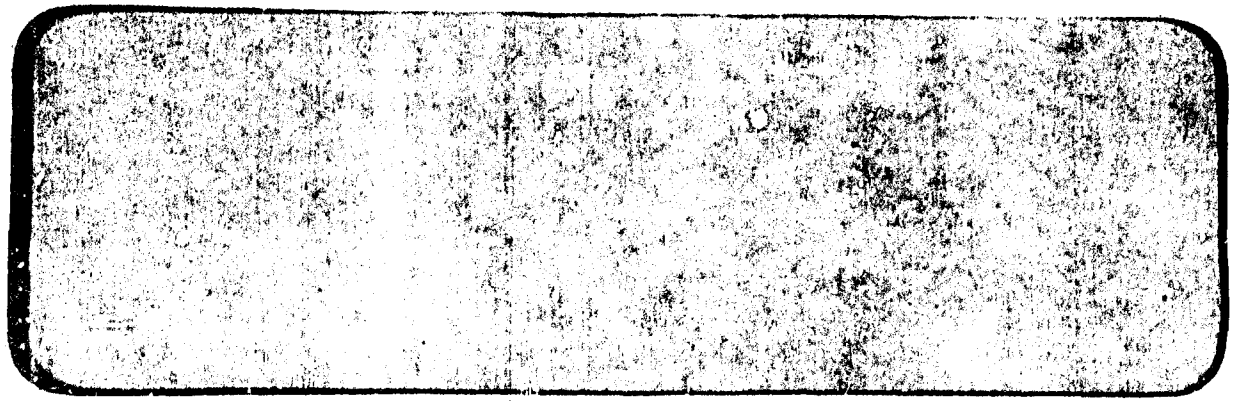

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Dependence of Time to Death on Molecular Size of Botulinum Toxin

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The molecular size of type A botulinum toxin affects the response in a time-to-death assay. Definitive statements of specific activity should be based on quantal assay.

The conventional quantal titration for botulinum toxin is based on the ratio of mice killed to the number injected at several dilutions in a period of 96 hr. A potentially simpler procedure depends on the time from challenge to death of relatively large doses of the toxin. In this case, the estimation of potency is made by interpolation from a predetermined time to death-dose response curve. This approach is attractive because a determination may be made in less than 2 hr. Such a method employing the intravenous route of injection into mice has been recommended (1) and employed for estimating the potency of a lower molecular weight [128,000 (2)] toxin derived from crystalline type A botulinum toxin (3), which has a molecular weight of 900,000 (7). It is our purpose to record evidence that questions the use of time to death as a standard index of toxic potency.

By repetition of the published method (1), data on survival times were obtained for both forms of the toxin. In Fig. 1, the decadic logarithms of the geometric means of the survival times versus the dose on a logarithmic scale in terms of LD_{50} for mice are plotted for three 10-fold dilutions of each toxin by both the intraperitoneal and intravenous routes of injection. The slopes, intercepts, and standard errors of the plots calculated by the analysis of variance are listed in Table 1.

It is clearly demonstrated that, for a given dose, the smaller molecule kills more rapidly than the larger molecule by both routes of injection, and the intraperitoneal route kills more slowly than the intravenous route. These findings preclude a rapid conversion of the large to the small molecule under physiological conditions, but they are consistent with the hypothesis that the time the toxin takes to escape from body fluids to reach specific receptor sites is influenced by molecular dimensions and the related property of diffusion rate. The dependence of the filtration mechanism of vessels of the circulatory systems on both the physical dimensions and intrinsic nature of protein molecules is well known. Escape of botulinum toxin from blood vessels is no exception (6).

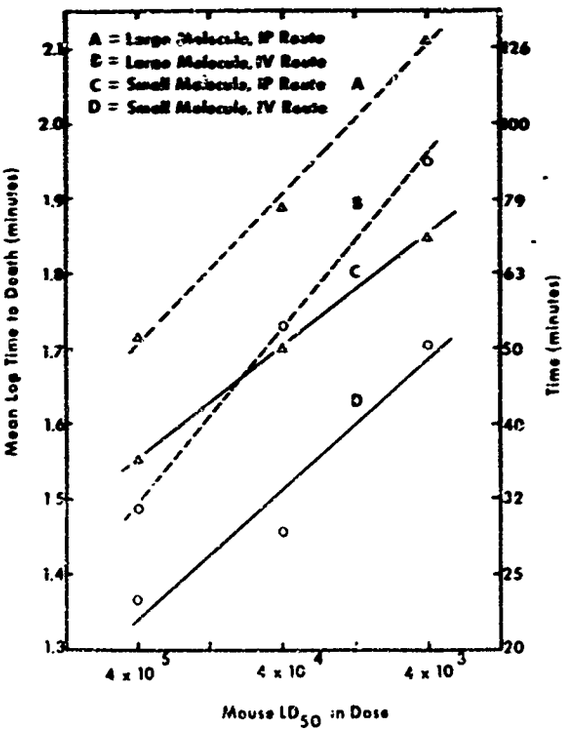

FIG. 1 *Plots of logarithm of average time to death against the dose on a logarithmic scale based on quantal assay of crystalline type A toxin and of a low molecular weight toxin derived from it. Intraperitoneal and intravenous routes of injection were studied, and each point represents the injection of 20 to 30 mice. Method of least squares was used to fit the curves.*

The slope and intercept of the time to death-dose response curve vary with the size of the toxin and the route of injection. The magnitude of these differences, however, was found to vary with

TABLE 1. *Standard error of slopes and intercepts of mortality time-dose response curves of type A botulinum toxin calculated by analysis of variance*

Toxin	Injection route	Slope	Standard error of slope	Intercept	Standard error of intercept
Crystalline	Intraperitoneal	0.19941	0.011394	1.30685	0.024503
	Intravenous	0.23210	0.010766	1.26267	0.014376
Low molecular weight	Intraperitoneal	0.15019	0.012586	1.40106	0.026901
	Intravenous	0.17175	0.014463	1.16779	0.032520

individual experiments. These variations could rest on a multitude of factors, e.g., strain of mice, human error, and differences in laboratory environment; they militate against the indiscriminate use of a standard curve.

These observations also point up the hazard in comparing specific activity of different states of the same toxin by interpolation from a curve derived from the study of one of these states. [Boroff and Fleck (1) suggest three mice as adequate for an accuracy of ±14%. This is questionable, since in a previous study (5) it was found that 420 mice would be required to achieve estimates within ±15% error and 30 mice would be required for estimates within ±60% error at 95% confidence limits by the intraperitoneal route.] The fivefold greater specific activity claimed for the type A toxin having a molecular weight of 128,000 (3) over that of crystalline toxin rests on such an interpolation. The specific activity of this material by the time of death assay was reported to be 18.6×10^7 LD_{50} per mg (4). In our studies, by quantal assay, the specific activity of this form of the toxin, based on 19 assays on 5 separate preparations of the material, was $10.5 \times 10^7 \pm 2.6 \times 10^7$ LD_{50} per mg, representing an increase in potency of about threefold. The facts and caution demand that authoritative statements of specific activity in comparing toxins be based on conventional quantal assays.

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